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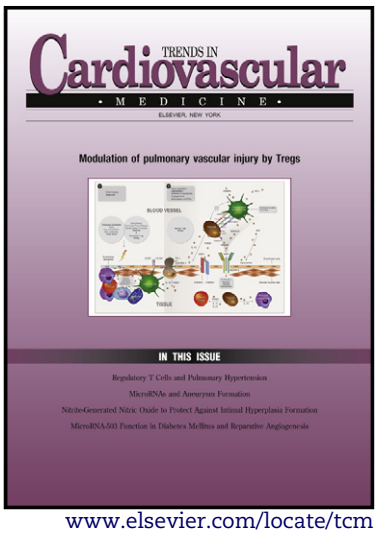

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A reliable marker of vascular function: does it exist?

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The major causes of mortality, and a great deal of morbidity, are cardiovascular disease and cancer. The endothelium, reputed to be the largest organ in the body (weighing about a kilogram and consisting of some 1-6 $x10^{13}$ cells (1), is undoubtedly the primary target for the disease process of atherosclerosis (2). Epidemiological studies such as the Framingham Heart Study and others have unequivocally defined the importance of the four major risk factors for this disease. Their pathological link is that, either directly or indirectly, each of the risk factors independently cause damage to the endothelium, and of course in a clinical setting they overlap as, for example, many diabetics also have hypertension and dyslipidaemia (3). As regards cancer, the endothelium is important because of its role in angiogenesis (4). Furthermore, the endothelium is sensitive to cytotoxic chemotherapy, and this is perhaps why some forms of chemotherapy are successful in that they preferentially destroy those blood vessels feeding a tumour. In both disease groups a damaged endothelium loses its anticoagulant nature and becomes procoagulant, thereby providing a link with atherothrombosis in cardiovascular disease, and potentially with the increased risk of venous thromboembolism in cancer (especially during bolus chemotherapy)(5). A malfunctioning endothelium is unable to part-regulate blood pressure, leading to hypertension. Loss of the barrier function of the endothelium seem likely to be a contributor to oedema, whilst the increased expression of adhesion molecules (such as intercellular adhesion molecule [ICAM] and E selectin which recruit leukocytes) and release of cytokines such as IL-6 are likely contributors to inflammation (6,7). Consequently, the endothelium is of great interest to oncologists, cardiologists and hematologists, all of whom are keen to develop methods of assessing the integrity of this tissue. Candidate methods include those of plasma markers, techniques based on blood flow, and of cell biology.

The endothelium secretes and/or releases and/or expresses at its cell surface a variety of molecules (table 1). These molecules have a variety of functions, such as contributing to the

regulation of hemostasis (when released or expressed luminally) and to vascular tone (when released into the vessel wall), some of which act as antagonistic pairs (7). Furthermore, several are easily measured in plasma by immunoassay, although not all are specific products of the endothelium. Endothelial integrity may also be assessed by changes in vascular tone, hypertension being a classic model, although endothelial-independent smooth muscle cell change may also be important in this disease. Nonetheless, endothelial function can be determined in a physiological setting by techniques such as flow mediated dilatation and arterial stiffness/pulse wave velocity, although these methods are slow and are strongly operator dependent (8-10).

The healthy endothelium adheres to the internal elastic lamina of the intima until it dies or is driven off by a disease process, at which time cells may the found in the plasma: hence circulating endothelial cells (CECs). Although described long ago (11,12), research on CECs took off once specific markers, such as CD146, were discovered (13). Thus armed, increased numbers of CECs were described in many cardiovascular, inflammatory and neoplastic diseases, the interpretation being that each disease process was (at least) partly responsible for this increase (14-16). However, others used alternative molecules to define CECs (17), and further confusion followed from the parallel discovery of bone marrow derived endothelial progenitor cells (EPCs), said to be a population that replaced the dead and dying CECs (18). Further confusion followed with the use of additional markers (many of which are expressed by non-endothelial cells (table 2)) such as CD34 and CD309 (19,20), and the use of intimately linked terms such as ‘circulating progenitor cell’ and ‘endothelial progenitor cells’, alone and in combination (21). The most recent development in this area is of endothelial microparticles (EMPs), exceptionally small particles of cytoplasm, increased numbers of which are, like CECs and plasma markers, increased in cardiovascular disease (22,23).

Schmidt et al have accurately summarised these issues in the present volume of the Journal (24).

Having agreed that the endothelium is an important organ/tissue whose status needs to be accessed, how should this be achieved? Clearly one of the more important arteries (if not the most important artery) is that of the epicardium, upon which the beating heart relies. Reminiscent of Koch's postulate for pathogenic organisms, Flammer et al (25), focussing on the heart, described nine criteria for an optimal endothelial function test, these being that it reflects the disease state, is reversible with interventions, mirrors coronary endothelial function, improves risk stratification, is reproducible, is operator independent, is non-invasive (with no or low risk for the patient), is easy to use and is inexpensive. Table 3 sets these nine criteria, and others, against a cross-section of methods (26). It is clear that none of the methods (as yet) comes anywhere near close to being a truly useful method, in the same way are the full blood count, urea and electrolytes and the electrocardiogram, for assessing coronary endothelial function. However, any of these methods may be useful in determining the state of other vascular systems, such as those of the brain. But in considering wider pathophysiological issues, an alternative use of these methods may be in determining global endothelial function and damage, and this may be important in other settings such as disseminated intravascular coagulation or in septicemia (27-30).

So using one or more of these tests, suppose we have identified a patient at high risk of an adverse cardiovascular event by virtue of poor endothelial function – how should we proceed? Inasmuch as the four major risk factors are cytotoxic to the endothelium, and that reversal of the factors restores endothelial integrity, then the strategy is clear. However, the process of treating the risk factors for atherosclerosis, whether by formal pharmaceutical intervention (statins, ACE inhibitors, hypoglycemics) or by simply adopting a healthy lifestyle (no smoking, a diet rich in fresh fruit and vegetables, regular exercise, avoidance of

overweight and obesity) has been known for decades as effective in reducing major cardiovascular events (31,32). Furthermore, vascular dysfunction is not the only pathophysiology that contributes to atherosclerosis. Suppression of platelet function by aspirin in probably the most successful single cardiovascular intervention of the 20th century, and in addition reversal of the risk factors is also likely to reduce inappropriate platelet and coagulation activation independently of any effect on the endothelium (33,34).

Clinical research is as sensitive to Darwinian mechanisms as any other field: our area of study is littered with disappointments, an excellent example being the hope of using viral plasmid as therapeutics (35). Similarly, endothelial progenitor cells have (as yet) not translated from the laboratory to the bedside (36), although more time may be needed. Two decades ago, I speculated that plasma markers may be useful in some settings, whilst a decade ago, Hwa et al drew attention to a bench-to-beside gap that has still to be closed (37,38). Although plasma von Willebrand factor adds to risk-factor scores for predicting outcome in atrial fibrillation (39), and despite its ease of measurement, much more work is required before even this one molecule is adopted as a routine laboratory marker. Although a daunting task, the introduction into routine pathology of brain naturietic peptide as marker of heart failure provides a model (40). However, perhaps our focus on one single marker is short-sighted. With an organ as complex and widespread as the endothelium perhaps a panel of markers representing different aspects of vascular physiology and pathology may be fruitful (7,41). Such a panel may well include CECs and/or endothelial microparticles as Schmidt and colleagues promote (24), but much work needs to be done, especially in the adoption of an international consensus on methodology.

References

1. Sumpio BE, Riley JT, Dardik A. Cells in focus: endothelial cell. Int J Biochem Cell Biol 2002;34:1508–12.
2. Mustard JF, Packham MA. The role of blood and platelets in atherosclerosis and the complications of atherosclerosis. Thromb Diath Haemorrh. 1975;33:444-56.
3. Grundy SM. Atherosclerosis: pathology, pathogenesis, and role of risk factors. Dis Mon. 1983;29:1-58.
4. Folkman J, Merler E, Abernathy C, Williams G. Isolation of a tumour factor responsible for angiogenesis. J Exp Med. 1971;133:275-88.
5. Blann AD. Endothelial cell activation markers in cancer. Thromb Res. 2012;129 Suppl 1:S122-6.
6. Giddings JC. Intercellular adhesion in vascular biology, thrombosis and cancer. Br J Biomed Sci. 1999;56:66-77.
7. Ramcharan KS, Lip GY, Stonelake PS, Blann AD. The endotheliome: a new concept in vascular biology. Thromb Res. 2011;128:1-7.
8. Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. J Physiol. 2005;568:357-69.
9. Hamilton PK, Lockhart CJ, Quinn CE, McVeigh GE. Arterial stiffness: clinical relevance, measurement and treatment. Clin Sci 2007;113:157-70.
10. Iantorno M, Weiss RG. Using advanced noninvasive imaging techniques to probe the links between regional coronary artery endothelial dysfunction and atherosclerosis. Trends Cardiovasc Med. 2014;24:149-56.

11. Bouvier CA, Gaynor E, Cintron JR, Bernhardt B, Spaet T. Circulating endothelium as an indication of vascular injury. Thromb Diath Haemorrh. 1970;40:163-168.
12. Hladovec J, Prerovsky I, Stanek V, Fabian J. Circulating endothelial cells in acute myocardial infarction and angina pectoris. Klin Wochenschr. 1978;56:1033–6.
13. George F, Poncelet P, Laurent JC, Massot O, Arnoux D, Lequeux N, Ambrosi P, Chicheportiche C, Sampol J. Cytofluorometric detection of human endothelial cells in whole blood using S-Endo 1 monoclonal antibody. J Immunol Methods. 1991;139:65-75.
14. Mutin M, Canavy I, Blann A, Bory M, Sampol J, Dignat-George F. Direct evidence of endothelial injury in acute myocardial infarction and unstable angina by demonstration of circulating endothelial cells. Blood. 1999;93:2951–8.
15. Woywodt A, Goldberg C, Kirsch T, de Groot K, Erdbruegger U, Haller H, Haubitz M. Circulating endothelial cells in relapse and limited granulomatous disease due to ANCA associated vasculitis. Ann Rheum Dis. 2006;65:164-8.
16. Mancuso P, Burlini A, Pruneri G, Goldhirsch A, Martinelli G, Bertolini F. Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. Blood. 2001;97:3658-61.
17. Mutunga M, Fulton B, Bullock R, Batchelor A, Gascoigne A, Gillespie JI, Baudouin SV. Circulating endothelial cells in patients with septic shock. Am J Respir Crit Care Med. 2001;163:195-200.
18. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997;275:964-7.
19. Quirici N, Soligo D, Caneva L, Servida F, Bossolasco P, Deliliers GL. Differentiation and expansion of endothelial cells from human bone marrow CD133(+) cells. Br J Haematol. 2001;115:186-94.

20. Schlingemann RO, Rietveld FJ, de Waal RM, Bradley NJ, Skene AI, Davies AJ, Greaves MF, Denekamp J, Ruiter DJ. Leukocyte antigen CD34 is expressed by a subset of cultured endothelial cells and on endothelial abluminal microprocesses in the tumor stroma. Lab Invest. 1990;62:690-6.
21. Costiniuk CT, Hibbert BM, Simard T, Ghazawi FM, Angel JB, O'Brien ER. Circulating endothelial progenitor cells in HIV infection: a systematic review. Trends Cardiovasc Med. 2013;23:192-200.
22. Chironi GN, Boulanger CM, Simon A, Dignat-George F, Freyssinet JM, Tedgui A. Endothelial microparticles in diseases. Cell Tissue Res. 2009 ;335:143-51.
23. Berezin A, Zulli A, Kerrigan S, Petrovic D, Kruzliak P. Predictive role of circulating endothelial-derived microparticles in cardiovascular diseases. Clin Biochem. 2015 Feb 16. pii: S0009-9120(15)00057-0. doi: 10.1016/j.clinbiochem.2015.02.003.
24. Schmidt DE, Manca M, Hoefer IE. Circulating endothelial cells in coronary artery disease and acute coronary syndromes. Trends Cardiovasc Med. 2015: In Press.
25. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, Hamburg NM, Lüscher TF, Shechter M, Taddei S, Vita JA, Lerman A. The assessment of endothelial function: from research into clinical practice. Circulation. 2012;126:753-67.
26. Lekakis J, Abraham P, Balbarini A, Blann A, Boulanger CM, Cockcroft J, Cosentino F, Deanfield J, Gallino A, Ikonomidis I, Kremastinos D, Landmesser U, Protogerou A, Stefanadis C, Tousoulis D, Vassalli G, Vink H, Werner N, Wilkinson I, Vlachopoulos C. Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. Eur J Cardiovasc Prev Rehabil. 2011:18:775-89.

27. Wada H, Minamikawa K, Wakita Y, Nakase T, Kaneko T, Ohiwa M, Tamaki S, Deguchi K, Shirakawa S, Hayashi T, et al. Increased vascular endothelial cell markers in patients with disseminated intravascular coagulation. Am J Hematol. 1993;44:85-8.

28. Okajima K, Uchiba M, Murakami K, Okabe H, Takatsuki K. Plasma levels of soluble E-selectin in patients with disseminated intravascular coagulation. Am J Hematol. 1997;54:219-24.

29. Delabranche X, Boisramé-Helms J, Asfar P, Berger A, Mootien Y, Lavigne T, Grunebaum L, Lanza F, Gachet C, Freyssinet JM, Toti F, Meziani F. Microparticles are new biomarkers of septic shock induced induced disseminated intravascular coagulopathy. Intensive care Med 2013:39;1695-703.

30. Skibsted S, Jones AE, Puskarich MA, Arnold R, Sherwin R, Trzeciak S, Schuetz P, Aird WC, Shapiro NI. Biomarkers of endothelial cell activation in early sepsis. Shock. 2013;39:427-32.

31. Blann AD, Lip GY. The endothelium in atherothrombotic disease: assessment of function, mechanisms and clinical implications. Blood Coagul Fibrinolysis. 1998:9:297-306.

32. Ceconi C, Fox KM, Remme WJ, Simoons ML, Bertrand M, Parrinello G, Kluft C, Blann A, Cokkinos D, Ferrari R. ACE inhibition with perindopril and endothelial function. Results of a substudy of the EUROPA study: PERTINENT. Cardiovasc Res. 2007;73:237-46.

33. Mehta P. Aspirin in the prophylaxis of coronary artery disease. Curr Opin Cardiol. 2002;17:552-8

34. Hennekens CH. Aspirin in the treatment and prevention of cardiovascular disease. Annu Rev Public Health. 1997;18:37-49.

35. Linden RM, Ward P, Giraud C, Winocour E, Berns KI. Site-specific integration by adeno-associated virus. Proc Natl Acad Sci U S A. 1996;93:11288-94.

36. Kawamoto A, Losordo DW. Endothelial progenitor cells for cardiovascular regeneration. Trends Cardiovasc Med. 2008;18:33-7.

37. Blann AD, Taberner DA. A reliable marker of endothelial cell dysfunction: does it exist? Br J Haematol. 1995:90:244-8.

38. Hwa C, Sebastian A, Aird WC. Endothelial biomedicine: its status as an interdisciplinary field, its progress as a basic science, and its translational bench-to-bedside gap. Endothelium. 2005;12:139-51.

39. Roldán V, Marín F, Muiña B, Torregrosa JM, Hernández-Romero D, Valdés M, Vicente V, Lip GY. Plasma von Willebrand factor levels are an independent risk factor for adverse events including mortality and major bleeding in anticoagulated atrial fibrillation patients. J Am Coll Cardiol. 2011;57:2496-504.

40. O'Donoghue M, Braunwald E. Natriuretic peptides in heart failure: should therapy be guided by BNP levels? Nat Rev Cardiol. 2010;7:13-20.

41. Aird WC. Endothelial cell heterogeneity. Crit Care Med. 2003;31(4 Suppl):S221-30.

Table 1: Products of the endothelium

Anti-coagulant/vasorelaxive Anti-inflammatory	**Pro-coagluant/vascoconstrictive Pro-inflammatory**
Nitric oxide	Endothelin
Prostacyclin	Thromboxane
Tissue plasminogen activator	Plasminogen activator inhibitor
Protein C	Tissue factor
Heparin	Von Willebrand factor
Thrombomodulin	Factor V
	Interleukins/cytokines
	Adhesion molecules

Table 2: Endothelial markers and their expression on non-endothelial cells

Marker	Antigen name	Expression on non-endothelial cells
PECAM-1	CD31	Platelets, leucocytes
ICAM-1	CD54	Leucocytes
Endoglin	CD105	Macrophages, activated monocytes, erythroid progenitors, pre-B lymphocytes
VCAM-1	CD106	Stromal cells, smooth muscle cells, fibroblasts
Thrombomodulin	CD141	Platelets, monocytes, neutrophils, keratinocytes
E-cadherin	CD144	Fetal liver cells
P1H12, S-endo-1	CD146	pericytes, bone marrow fibroblasts, nerve fibres, activated T-lymphocyte, malignant cells
VEGF receptor 1, KDR	CD309	Hematopoietic cells, progenitor cells
von Willebrand factor		Platelets

Table 3: Criteria for an Optimal Endothelial Function Test

Criterion	Plasma markers *	CECs	EMPs	FMD	PWV/AS	Coronary epicardial vasoreactivity
Reflects disease state	Probably	Possibly	Possibly	Probably	Probably	Yes
Reversible with interventions	Yes	Unclear, probably	Possibly	Yes	Probably	Yes
Reflects coronary endothelial function	No	No	No	Indirectly	Possibly	Yes (Gold Standard)
Improves risk stratification	Possibly	Unclear	Possibly	Possibly	Possibly	Probably
Reproducibility	Good	Poor	Moderate /poor	Moderate	Moderate	Moderate
Operator independent	Yes	Yes	Yes	No	No	No
Invasive	No	No	No	No (but inconvenient	No	Very
Easy to use	Yes	No	No	No	No	No
Inexpensive	Yes	No	No	No	No	No
Consensus on definition	Yes	Weak	Weak	Yes	Yes	Yes
Consensus on method	Yes	No	Weak	Modest	Modest	Yes
Potential as a Global marker	Yes	Yes	Yes	Possibly	Possibly	No

*for example, von Willebrand factor, soluble thrombomodulin. CECs = circulating endothelial cells, EMPS = endothelial microparticles, FMD = flow mediated dilatation, PWV/AS = pulse wave velocity/ arterial stiffness.